IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s)	:	Pasternak et al.
Serial No.	:	09/975,812
For	:	TOPICAL ANESTHETIC/OPIOID FORMULATIONS AND USES THEREOF
Filed	:	October 11, 2001
Examiner	:	Bahar
Art Unit	:	1617

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I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" Service under 37 CFR 1.10 on the date indicated above and is addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Charles B. Jackson
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(Signature of person mailing paper or fee)

DECLARATION OF DR. SANDRA C. ROERIG UNDER 37 C.F.R. § 1.132

I declare as follows:

1. I am an associate editor of the editorial board of the Journal of Pharmacology and Experimental Therapeutics. I am familiar with U.S. Application Serial No. 09/975,812. I have been informed that U.S. Application Serial No. 09/975,812 was filed on October 11, 2001, claiming priority to 09/844,111, filed on April 27, 2001 and U.S. Provisional Application Serial No. 60/200,437, filed April 28, 2000. My curriculum vite is provided under Tab 1. I respectfully submit that I am qualified to speak and render opinions as to the

disclosure in the present application, the state of the art and the procedures of editorial review at the Journal of Pharmacology and Experimental Therapeutics. Furthermore, I have reviewed the experimental work discussed herein, in the ordinary course of business.

2. I am familiar with the Office Action dated February 26, 2003, issued by the United States Patent and Trademark Office in connection with the present application and make this Declaration in response thereto. I will address the following issue to respond to the Examiner's rejections:

> The role of peripheral mechanisms in the mediation of antinociceptive responses was unknown prior to the teaching of the present invention. Opioid analgesia was thought to be mediated through the central nervous system (i.e. systemically) rather than through peripheral opioid receptors. Those skilled in the art did not appreciate the significance of peripheral opioid receptor stimulation, much less the significance of combining opioid analgesics and local anesthetics at these peripheral sites. The synergistic potentiation of pain relief that occurs at peripheral sites when opioid analgesics are administered together with local anesthetics was unexpected, especially given that only small amounts of each drug are needed to produce a synergistic response.

3. Details of the editorial review process are described herein. The Journal of Pharmacology and Experimental Therapeutics invites for review original papers dealing with interactions of chemicals with biological systems. All aspects of pharmacology and therapeutics are appropriate. The American Society for Pharmacology and Experimental Therapeutics, which the journal is a member of, requires authors to affirm that original studies reported in the journals of the Society have been carried out in accordance with the Declaration of Helsinki and/or with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health.

4. At least two independent reviewers, skilled in the art, are selected for each submitted manuscript. The review is blinded such that the two selected reviewers are unaware of each

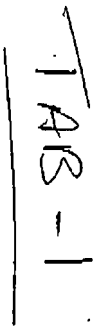
other. Comments to the author are intended to be constructive without indicating acceptability of the manuscript. Based substantially on the reviewers' comments, the Associate Editor makes a decision to accept or deny the manuscript for publication. A copy of the reviewers' comments for authors Drs. Yuri Kolesnikov, Igor Chershnev, and Gavril W. Pasternak in response to the manuscript entitled "Analgesic Synergy between Topical Lidocaine and Topical Opioids", is provided under Tab 2. A copy of the manuscript in its published form is provided under Tab 3. To the best of my knowledge, the data reviewed and described in the publication is the same as in the present application.

5. The present invention is directed to topical administration of morphine and lidocaine, which together produce a synergistic antinociceptive response in the periphery. The position of our reviewers was that the synergistic effect of topical morphine and lidocaine at the amounts used was "profound" and "quite marked." Essentially, their position was that the result was unexpected. In addition, one of the reviewers noted that studies of this kind had "never been performed previously." These statements, dated May 19, 2000, provide evidence of the state of the art, from those skilled in the art, at the time the instant application was filed.

6. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful statements may jeopardize the validity of the application or any patent issued thereon.

Dated 5/12/03

By: Sandra C Roenig
Sandra C. Roenig



CURRICULUM VITAE

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EDUCATION

B.S., Horticulture, Kansas State University, 1967

M.S., Pharmacology, Medical College of Wisconsin, 1976

Ph.D., Pharmacology, Medical College of Wisconsin, 1988

EXPERIENCE

2001-present	Associate Dean for Research and Graduate Studies Louisiana State University Health Sciences Shreveport, LA
July 2002-present	Professor, Department of Pharmacology and Therapeutics Louisiana State University Health Sciences Center Shreveport, LA
July 2002-present	Professor, Department of Anesthesiology Louisiana State University Health Sciences Center Shreveport, LA
2000-2001	Assistant Dean, School of Graduate Studies Louisiana State University Health Sciences Shreveport, LA
1997-2002	Associate Professor, Department of Pharmacology and Therapeutics Louisiana State University Health Sciences Center Shreveport, LA
1991 - 1997	Assistant Professor, Department of Pharmacology and Therapeutics Louisiana State University Medical Center Shreveport, LA
1989-1991	Postdoctoral Fellow, Department of Pharmacology University of Minnesota, Minneapolis, MN Advisor: Dr. Horace H. Loh

1988 -1989	Postdoctoral Fellow, Department of Pharmacology University of Minnesota, Minneapolis, MN Advisor: Dr. George L. Wilcox
1984 - 1987	Graduate Student, Department of Pharmacology and Toxicology Medical College of Wisconsin, Milwaukee, WI Advisor: Dr. James M. Fujimoto
1976-1984	Research Associate, Department of Pharmacology and Toxicology Medical College of Wisconsin, Milwaukee, WI Supervisor: Dr. James M. Fujimoto
1975-1976	Graduate Student, Department of Pharmacology and Toxicology Medical College of Wisconsin, Milwaukee, WI Advisor: Dr. James M. Fujimoto
1972-1975	Research Technician, Department of Pharmacology Medical College of Wisconsin, Milwaukee, WI Supervisor: Dr. James M. Fujimoto
1969-1971	Research Technician, Biochemistry, ABC Plant Research Lab Michigan State University, East Lansing, MI Supervisor: Dr. Derek T.A. Lamport
1967-1969	Research Technician, Department of Biochemistry University of Kansas Medical Center, Kansas City, KS Supervisor: Dr. Dennis Diedrich, Dr. Santiago Grisolia
1965-1967	Research Technician, Horticulture, School of Agriculture Kansas State University, Manhattan, KS Supervisor: Dr. William Carpenter

SOCIETY MEMBERSHIPS

American Society for Pharmacology and Experimental Therapeutics
American Society for the Advancement of Science
Society for Neuroscience
American College of Clinical Pharmacology (Fellow)
International Narcotics Research Council

AWARDS

Tuition Scholarship, Medical College of Wisconsin, Graduate Studies Council, (1985-1986)
Travel Award, American Society for Pharmacology and Experimental Therapeutics, (1986)
Travel Award, Friends of Medical College of Wisconsin (1987)
Travel Award, Committee on Problems of Drug Dependence (1988)
Travel Award, American College of Neuropsychopharmacology (1988)

TEACHING

Student Conferences, General Pharmacology, Medical College of Wisconsin (1984-1987)

Lectures, General Pharmacology, School of Nursing, Medical College of Wisconsin (1984)

Teaching Assistant, Neuroscience Summer Workshop, Lake Itasca, University of Minnesota, (1988)

Medical Pharmacology Lectures and Student Conferences,
LSU Health Sciences Center (1991-present)

Clinical Pharmacology Conferences,
LSU Health Sciences Center (1992 - 1999)

Lectures in Graduate level courses:
Principles of Pharmacology I and II, Neurochemistry, Philosophical and Ethical Issues in Science, Behavioral Pharmacology, Neuropharmacology, Molecular Pharmacology, Integrative Structural Biology, Fundamentals of Biological Sciences
LSU Health Sciences Center (1992- present)

Course Director:
Principles of Pharmacology I, (1993-1996) Molecular Pharmacology (1996-2000) Clinical Pharmacology Conferences (1993 - 1998), LSU Health Sciences Center

Joint LSU/HSC Physiology Department-Centenary College Summer Seminar Series
Lectures in Mentoring to undergraduate students (1996-present)

GRADUATE EDUCATION

Postdoctoral Fellows

Natalie Lenard, Ph.D., 2003-present

Guoqiang Gutan, D.D.S., Ph.D., 2000-2002

Department of Pharmacology LSU Health Sciences Center Graduate Students

Research Advisor for: Zhong Yon Wei, Yaohui Li, Farizana Karim, Laura Tédesco, Scott Baker

Dissertation/Thesis committee member for: Ying Ye, Kehong Zhang, Pankaj Sikka, Donna Ross, James Hinson, Orlando Bacon, Alicia Christman, Yu Zhao, Troy Casac, Olga Gurkovskaya, Rachell Kommajfi

Students Graduated:

Zhong You Wei, M.S., 1995
Thesis title: Vertigo-dependent calcium channels and G proteins in spinal morphine/clonidine synergistic antinociception

Yanhui Li, M.S., 1997
Thesis title: Alterations of Spinal Protein Kinase C Expression and Kinetics in Morphine Tolerance

Farzana Karim, Ph.D. 1999
Dissertation title: Functional aspects of opioid and alpha$_2$ adrenergic receptor activation: involvement of specific G proteins

Medical Student Summer Research Program

Students mentored:
Job Broyles (1993)
Eric Madore (1994)
Matthew Chamberlain (1996)
Joan Cheak (1999)

Undergraduate and Teacher Summer Research Program

Students Mentored:
Lisa Walker (1994)
Chancy Burden (1998)
Kavita Behar (1997)

Multicultural Affairs "Jump-Start Program" for High School Students

Students Mentored:
Deana Rambo (summer 2000)

Other Student Related Activities

Department of Pharmacology and Therapeutics Graduate Student Coordinator (1997-2000)

Organized LSUHSC-Shreveport Graduate Student Orientation (2000)

GRANT SUPPORT

Awarded as Principle Investigator

National Institute on Drug Abuse, Research Fellowship Award DA 05370 (Oct. 1, 1988-Sept. 30, 1991) - "Partial Characterization of Cloned Delta (Opioid Receptor"

The Edward P.S. Stiles Trust Fund - LSUMC-S Institutional Funds, Young Investigator Award (Nov. 1, 1991 - Oct. 31, 1992) "Spinal Opioid and Adrenergic Analgesia in Opioid Tolerance" - $7,450. Renewed (Dec. 1, 1992 - Nov. 30, 1993) - $7,462

American Cancer Society Junior Investigator Award, Institutional support (May 1, 1992-June 30, 1993) "Identification of GTP-binding proteins which transduce spinal opioid receptor functions" - $6,000

Louisiana Education Quality Support Fund (July 1, 1993 - June 30, 1996) "Second messenger systems involved in opioid and alpha adrenergic interactions" - $144,976 - Approved

National Institutes on Drug Abuse, FIRST Award (May 1, 1993 - April 30, 1998) "Opioid and Alpha Adrenergic Agonist Interactions" - DA07972-$350,018

The Edward P.S. Stiles Trust Fund - LSUMC-S Institutional Funds, Bridging Award, "Opioid and Alpha Adrenergic Agonist Interactions" (January 1, 1999-December 30, 1999, $30,000)

National Institutes on Drug Abuse, R03, DA12547, "Spinal nitric oxide in chronic inflammatory pain" (1/1/00-12/31/01) $100,000

Awarded as Contract for Program Project

National Institutes on Drug Abuse, Program Project, "Design of opioid analgesics devoid of tolerance/addiction", PI, Ping Law, University of Minnesota (6/1/02-5/30/07) $346,926

Awarded as Co-Investigator

National Institutes of Child Health and Development, RFA 9306, Pediatric Drug Evaluation Resource (9/30/93-9/30/98) Principle Investigator, John Wilson, M.D., Efficacy and pharmacokinetics of tramadol for treatment of pain in children, Sandra C. Ronzio, Basic Investigator - $1,600,000

Submitted, October 1, 2001

National Institutes on Drug Abuse RO1 - "Spinal nitric oxide in chronic inflammatory pain" for 7/1/02-6/30/06, $500,000, not funded, will be resubmitted

SERVICE

GRANT REVIEWER

National Grant Reviews:

Study Sections:

ad hoc reviewer for SBIR applications, Molecular Biology Section - July 1998

ad hoc reviewer for IFCN-4, National Institutes of Health, October 14-16, 1998

ad hoc reviewer for NIH IFCN-7, SBIR Study section - April 2000, August 2001, March 2002, April 2003

Phone Reviews:

ad hoc reviewer for NIH (Thalamin) - October 1993

ad hoc reviewer for intramural grant at Allegany College, PA , 1997

ad hoc reviewer for EPSCoR grant application, March 1998

ad hoc reviewer for NIH IFCN-4, December 1998

Special Grant Reviewer for NIH, October 1995, December 1998, March 1999

ad hoc reviewer and chair of IFCN5-03 Study Section - October 2000

ad hoc reviewer for IFCN2 - December 2001

LSUHSC COMMITTEE SERVICE

1. Department of Pharmacology

1992, 1994, 2000	Pharmacology Faculty Search Committee	Member
1993, 1996, 2000	Qualifying Exam Committee	Member
1994	Faculty review of USMLE Step 1 (Nov. 17, 1993)	Member

2. LSUHSC - Shreveport

1994	Search Committee for Head, Dept. of Neurology	Member
1993-1997	Radiation Safety Committee	Member
1996- 2001	Radiation Safety Committee	Chair
1997-1999, present	Admissions Committee	Member
1996	Reviewer of Cancer Center Applications	Member
1995-1998	Elected Faculty Council	Member
1997-1998	Elected Faculty Council	Chair
1995-1997, 2000	Research Advisory Committee	Member
1997-1999	Radioactive Drug Research Committee	Member
1998-1999	LCMB Visit Preparation Committee	Member
1999	Clinical Research Committee	Member
1999-present	Committee on Committees	Member
1999-present	Curriculum Committee	Member
2000-present	Committee to Draft Faculty Senate Bylaws	Member

3. LSUHSC - Faculty Senate for both campuses, Shreveport and New Orleans

1997-2001	LSUHSC - Shreveport Graduate School Representative	Member
1997-2001	Subcommittee for Faculty Welfare	Member
1999-2001	Representative to the Board of Supervisors	
2000-2001		Chair-elect

NATIONAL COMMITTEES

American Society for Pharmacology and Experimental Therapeutics
Subcommittee for Women in Pharmacology (1994-present)
Committee for Division of Education (2000-present)

Steering committee for 4th International Symposium on Imidazoline/Agmatine Systems
2001 - present

OTHER SERVICE

Director, Department of Pharmacology Seminar Program: LSU Medical Center (1993-1995)

Assistant Dean, School of Graduate Studies, LSUHSC-Shreveport, October 2000 - present

INVITED SEMINARS

Louisiana State University

1. Medical Center in Shreveport campus

Department of Cell Biology and Anatomy - 1992

Pathophysiology of Pain Symposium - 1993

Department of Neurology Grand Rounds - 1995

Clinical Pharmacology Interest Group -1996

Department of Molecular and Cellular Physiology -1998

2. Shreveport campus (undergraduate)

Seminars for the Department of Biology (1992-1996, 1999, 2001)

National

Department of Pharmacology, University of Texas Medical Center, Houston, TX (1993)

Department of Physiology, University of North Texas Health Sciences Center, Fort Worth, TX (1993)

Department of Pharmacology, University of Wisconsin - Madison, Madison, WI (1994)

Department of Pharmacology, Michigan State University, East Lansing, MI (1997)

Department of Pharmacology, University of Houston School of Pharmacy - Houston, TX (2000)

Department of Pharmacology, University of Arkansas Medical School - Little Rock, AR (2000)

OTHER PRESENTATIONS

June 7, 1997, Role of Protein Kinases in Spinal Morphine/Clonidine Antinociceptive Synergism, Pain Interest Group Meeting, Milwaukee, WI

CONTRIBUTIONS TO REFEREED PUBLICATIONS

1999 - present - Associate Editor, *Journal for Pharmacology and Experimental Therapeutics*

1994-1998 - Editorial Advisory Board, *Journal for Pharmacology and Experimental Therapeutics*

1995 - present - Editorial Board, *Analgesia*

1996-present - reviewer for *Brain Research, Journal of Neurochemistry, Life Sciences, Brain Research Bulletin, Peptides, Proceedings of the Society for Experimental Biology and Medicine, Journal for Pharmacology and Experimental Therapeutics, Journal for Neuroscience, Pain, Free Radical Biology and Medicine, Neurochemistry International*

PUBLICATIONS

Roerig, S., Fujimoto, J.M., Wang, R.I.H., Isolation of hydromorphine and dihydromorphine glucuronides from urine of the rabbit after hydromorphine administration. *Proc. Soc. Expt. Biol. Med.* 143: 230-233 (1973)

Chatterjie, N., Fujimoto, J.M., Iuturisi, C.E., Roerig, S., Wang, R.I.H., Bowen, D., Field, F.H., and Clarke, D.D., Isolation and sterochemical identification of a metabolite of naltrexone from human urine. *Drug Metab. Disp.* 2: 401-405 (1974)

Fujimoto, J.M., Roerig, S., Wang, R.I.H., Chatterjie, N. and Iuturisi, C.E., Narcotic antagonist activity of several metabolites of naloxone and naltrexone tested in morphine dependent mice. *Proc. Soc. Expt. Biol. Med.*, 148: 443-448 (1975)

Lampert, D.T.A., Katona, L. and Roerig, S., Galactosylserine in extensin. *Biochem. J.*, 133: 125 (1976)

Roerig, S., Fujimoto, J.M., Wang, R.I.H., and Lange, D.G., Preliminary characterization of enzymes for reduction of naloxone and naltrexone in rabbit and chicken liver. *Drug Metab. Disp.* 4: 53-58 (1976)

Roerig, S.C., Fujimoto, J.M., and Wang, R.I.H., The stimulatory effect of morphine on metabolism of naloxone to 6α-naloxol in the guinea pig. *Drug Metab. Disp.* 5: 434-463 (1977)

Roerig, S.C., Fujimoto, J.M. and Wang, R.I.H., The stimulatory effect of morphine on reduction of naltrexone to 6α-naltrexol in the guinea pig. *Drug Metab. Disp.* 8: 295-299 (1980)

Roerig, S.C., Christiansen, K.L., Jansen, M.A., Wang, R.I.H., Fujimoto, J.M., and Nickerson, M., Phylogenetic distribution of the hepatic enzyme system for reducing naloxone to 6α- and 6β-naloxol in vertebrates. *Comp. Biochem. Physiol.* 15: 93-97 (1980)

Lange, D.G., Roerig, S.C., Fujimoto, J.M. and Wang, R.I.H., Absence of cross-tolerance to heroin in morphine tolerant mice. *Science* 208: 72-74 (1980)

Lange, D.G., Roerig, S.C., Fujimoto, J.M. and Wang, R.I.H., Enhancement of morphine brain concentrations and changes in morphine-naloxone pA_2 values in morphine pretreated mice. *Biochem. Pharm.* 30: 147-155 (1981)

Lange, D.G., Roerig, S.C., Fujimoto, J.M. and Busse, L.W., Withdrawal tolerance and unidirectional non-cross tolerance in narcotic pellet implanted mice. *J. Pharmacol. Exp. Therap.*, 224: 13-20 (1983)

Brown, C.E., Roerig, S.C., Fujimoto, J.M. and Burger, V.T., The structure of morphine differs between the crystalline state and aqueous solution. *J. Chem. Soc., Chem Commun.* 1506-1508 (1983)

Roerig, S.C., O'Brien, S.M., Fujimoto, J.M. and Wilcox, G.L., Tolerance to morphine analgesia: decreased multiplicative interaction between spinal and supraspinal sites. *Brain Res.* 308: 360-363 (1984)

Brown, C.E., Roerig, S.C., Burger, V.T., Cody, R.B. and Fujimoto, J.M., Analgesic potencies of morphine 3- and 6-sulfates after intracerebroventricular administration in mice: relationship to structural characteristics defined by mass spectrometry and nuclear magnetic resonance, *J. Pharm. Sci.*, 74: 821-824 (1984)

Roerig, S.C., Fujimoto, J.M., Franklin, R.B. and Langs, D.G., Unidirectional non-cross tolerance (UNCT) in rats and an apparent dissociation between narcotic tolerance and physical dependence. *Brain Res.* 327: 91-96 (1985)

Roerig, S.C., Fujimoto, J.M. and Lange, D.G., Development of tolerance to respiratory depression in morphine- and etorphine-pellet-implanted mice. *Brain Res.*, 400: 278-284 (1987)

Roerig, S.C., Artean, C. and Fujimoto, J.M., Antagonism by naltrexone of systemic and intrathecal morphine-induced analgesia in mice. *Proc. Soc. Expt. Biol. Med.*, 186: 234-239 (1987)

Roerig, S.C., Fujisoto, J.M. and Tseng, L.F., Comparisons of descending pain inhibitory pathways activated by β-endorphin and morphine as characterized by supraspinal and spinal analgesic interactions in mice. *J. Pharmacol. Exp. Ther.* 247: 1107-1113 (1988)

Roerig, S.C. and Fujimoto, J.M., Morphine analgesia in different strains of mice: relationship of supraspinal-spinal multiplicative interaction to tolerance. *J. Pharmacol. Exp. Ther.*, 247: 603-608 (1988)

Roerig, S.C. and Fujimoto, J.M., Multiplicative interaction between intracerebroventricularly and intrathecally administered morphine for analgesia in mice: involvement of mu, delta and kappa receptors. *J. Pharmacol. Exp. Ther.* 249: 762-768 (1989)

Kady, Jeche J., Roerig, Sandra C. and Fujimoto, James M., Heroin acts on different opioid receptors than morphine in Swiss Webster and ICR mice to produce antinociception, *J. Pharmacol. Exp. Ther.* 256: 448-457 (1991)

Roerig, S.C., Hoffman, R.G., Takemori, A.E., and Fujimoto, J.M., Isobolographic analyses of analgesic interactions between intracerebroventricularly and intrathecally administered opioid agonists: morphine, fentanyl and D-Ala^2-D-Leu^5-enkephalin. *J. Pharmacol. Exp. Ther.*, 257: 1091-1099 (1991)

Roerig, Sandra C., Loh, H.H. and Law, P.Y., Requirement of ADP-ribosylation for the pertussis toxin-induced alteration in electrophoretic motility of G-proteins. *Biochem. Biophys. Res. Comm.*, 180:1227-1232 (1991)

Roerig, S.C., Lei, S. Kitto, K. Hylden, J.K.L. and Wilcox, G. L., Interactions between spinally administered opioid and noradrenergic agonists in the substance P test in mice: multiplicitivity involves δ and alpha2 receptors. *J. Pharmacol Exp. Ther.*, 262: 365-374 (1992)

Roerig, Sandra C., Law, P.Y. and Loh, H.H., Identification of three separate guanine nucleotide-binding proteins which interact with the δ opioid receptor in NG108-15 neuroblastoma x glioma hybrid cells. *Mol. Pharm.*, 41: 822-831 (1992)

Dujic, Z., Manijic, J., Roerig, S. C., Dujic, I., Kampine, J. P. and Bosnjak, Z., Presynaptic modulation of acetylcholine release from the cat stellate ganglion by morphine, *Croatian Med. J.*, 34: 33-42 (1993)

Sapbner, D., Roerig, S.C., Ito, C., Viasak, W.R., Parrar, G.E., Broyles, J.E. and Welch, J.E., Inhibition of neural and adrenocortical activity by α-interferon: neuroendocrinic, electrophysiological and biochemical studies in the rat, *Brain, Behav Immun.*, 8:37-56 (1994)

Roerig, Sandra C., Decreased spinal morphine/clonidine antinociceptive synergism in morphine-tolerant mice, *Life Sci.*, 56: PL115-PL122 (1995)

Roerig, Sandra C., Cynthia L. Williams, Victor J. Hruby, Thomas F. Burks and Gary Rosenfeld, Inhibition of adenylyl cyclase activity by the cholecystokinin analog SNF90007 in neuroblastoma x glioma NG108-15 hybrid cells, *Reg. Peptides*, 61: 51-56 (1996)

Wei, Zhong you, Farzana Karim and Sandra C. Roerig, Spinal morphine/clonidine antinociceptive synergism: involvement of G proteins and N-type voltage-dependent calcium channels, *J. Pharm. Exp. Therap.* 278:1392-1407 (1996)

Roerig, Sandra C. and Kurt Howse, ω-Agatoxin IVA blocks spinal morphine/clonidine antinociceptive synergism, *Eur. J. Pharmacol.*, 314:293-300 (1996)

Wei, Zhong you and Sandra C. Roerig, Spinal morphine/clonidine antinociceptive synergism is regulated by protein kinase C but not protein kinase A activity, *J. Pharmacol. Exp. Therap.* 287:937-943 (1998)

Li, Yaohui and Sandra C. Roerig, Alteration of Spinal Protein Kinase C Expression and Kinetics in Morphine, but not Clonidine Tolerance, *Biochem Pharmacol.*, 58:493-501 (1999)

Roerig, Sandra C., Timothy Brasch and Yaohui Li, Decreased spinal morphine/clonidine antinociceptive synergism in clonidine-tolerant mice, *Analgesia*, 4:187-195 (1999)

Napier, Leslie D., Sandra C. Roerig, Daine A. Yoshishige, Barbara A. Barron and James L. Caffrey, Canine cardiac muscarinic receptors, G-proteins and adenylate cyclase following chronic morphine, *J. Pharmacol. Exp. Ther.*, 291: 725-732 (1999)

Zavecz, James, H., Harold D. Baltarbee, Orlando Bueno, Ronald E. Maloney, Sandra C. Roerig and James M. O'Donnell, Down regulation of cardiac L-type Ca^{2+} channels in the portal hypertensive rat *Amer. J. Physiol.*, 279:G28-G39 (2000)

Kanfin, Farzana and Sandra C. Roerig, Differential effects of antisense oligodeoxynucleotides directed against $G_{z\alpha}$ and $G_{o\alpha}$ on antinociception produced by spinal opioid and α_2 adrenergic receptor agonists (*Pain*, 87 181-191, 2000)

Tedesco, Laura, John Fuseler, Matthew Grisham, Robert Wolf and Sandra C. Roerig, Nitric oxide synthase inhibitors reverse hyperalgesia but not inflammation in a rat model of chronic arthritis *Pain*, 95: 215-223, 2002

Roerig, Sandra C., Spinal and supraspinal agmatine activate different receptors to enhance spinal morphine antinociception (submitted, *J. Pharmacol. Exp. Ther.*)

Karim, Farzana, Paul Prather and Sandra C. Roerig, Opioid and alpha2-adrenergic receptor agonists enhance incorporation of [α-^{32}P] GTP azidoanilide into spinal G proteins (submitted, *Biochem. Pharm.*)

ABSTRACTS

Lamport, D.T.A., Katona, L. and Roerig, S., Amino acid sequence of hydroxyproline-rich tryptic peptides from acid-stripped primary cell walls. *Fed. Proc.* 30: 1317 (1971)

Lange, D.G., Fujimoto, J.M., Roerig, S.C. and Wang, R.I.H., Morphine induced sensitization to naloxone: enhanced disposition of naloxone to the brain. *The Pharmacologist*, 16: (1974)

Lange, D.G., Fujimoto, J.M., Roerig, S. and Wang, R.I.H., The Effect of morphine on the metabolism of naloxone. National Drug Abuse Conference (1976)

Fujimoto, J.M., Roerig, S.C. and Wang, R.I.H., Reduction of naloxone by the guinea pig. *Fed. Proc.* 34: 487 (1976)

Roerig, S., Fujimoto, J.M., Nickerson, M. and Wang, R.I.H., A preliminary comparison of the liver enzyme systems for reducing naloxone to 6α-and 6β-naloxol. Abstracts of Papers, Soc. of Toxicol., 15th Annual Meeting, 87 (1976)

Roerig, S., Fujimoto, J.M. and Wang, R.I.H., Simulation of morphine on metabolism of naloxone to 6α-naloxol in the guinea pig. *The Pharmacologist*, 18: 131 (1976)

Roerig, S.C., Fujimoto, J.M. and Wang, R.I.H., Effect of morphine on naltrexone metabolism in the guinea pig. *The Pharmacologist*, 20: 346 (1978)

Fujimoto, J.M., Lange, D.G., Roerig, S.C. and Wang, R.I.H., Development of differential tolerance to narcotic agonists by etorphine pellet implantation in mice. *The Pharmacologist*, 21: 79 (1979)

Roerig, S.C., Fujimoto, J.M. and Lange, D.G., Separation of expression of narcotic tolerance from physical dependence in rats. *The Pharmacologist*, 21:439 (1976)

Lange, D.G., Fujimoto, J.M., and Roerig, S.C., Continuous intraventricular infusions of narcotic agonists does not alter analgesic potency of systemically administered narcotics. *The Pharmacologist*, 21: 440 (1976)

Roerig, S.C., Lange, D.G., Wang, R.I.H., and Fujimoto, J.M., Differences in withdrawal tolerance ED50 values for several narcotics in etorphine pelleted mice. *The Pharmacologist*, 22: 786 (1980)

Roerig, S.C., Lange, D.G., and Fujimoto, J.M., Comparison of tolerance to respiratory depression from morphine (M), etorphine (E) and heroin (H) in morphine pellet implanted mice. *The Pharmacologist*, 23: 115 (1981)

Roerig, S.C., Lange, D.G. and Fujimoto, J.M., Tolerance to the potent narcotic analgesic morphine-6-ethereal sulfate (M6ES) in mice. *The Pharmacologist*, 24: 126 (1982)

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L. Tedesco, J. Fuseler, M. Grisham, R. Wolf and S. Roerig, Inhibition of nitric oxide synthase (NOS) activity reverses thermal hyperalgesia but not mechanical allodynia or inflammation in peptidoglycan/polysaccharide (PG/PS)-induced polyarthritis, Southeastern Pharmacology Society meeting, August, 2000.

Guoqiang Guan, Scott Baker, and Sandra C. Roerig., Inhibition of Spinal Nitric Oxide Synthase (NOS) Activity Reverses Carrageenan (CARRA)-induced Hyperalgesia But Not Inflammation In Rats, Experimental Biology, 2002

TAB-2

The Journal of Pharmacology and Experimental Therapeutics

COMMENTS FOR AUTHOR

To Reviewer #1
Associate Editor: Sandra C. Roerig, Ph.D.
Manuscript (MS) #: JPET#2090/3025
MS Title: Analgesic Synergy between Topical Lidocaine and Topical Opioids
Authors: Yuri A. Kolesnikov, Igor Chereshnev, and David W. Pasternak

INSTRUCTIONS FOR REVIEWER

1. When you have completed your review, please divide your comments into:
   a) Comments for Editor - Please type directly onto the pink sheet.
      These comments will not be sent to the author.
      Comments regarding anonymity must be confined to the pink sheet.
   b) Comments for Author - Please type directly onto the yellow sheet.
      These comments should be constructive without revealing anonymity of the reviewer.

COMMENTS: Date Reviewed: 8/16/07

The manuscript entitled "Analgesic synergy between topical lidocaine and topical opioids" (JPET#2090/3025) by Kolesnikov et al. describes the potential for further development of topical combination therapy for pain relief. The primary emphasis of this manuscript is the observed absence of side effects that can be problematic with most other forms of opioid administration. The synergistic effects were so profound, one wonders if these side effects really would persist under clinical conditions and with repeated use. It would be nice if there were some discussion of this in the discussion as it might be the Achilles heel of a really nice drug. Obviously, there questions won't be solved in these studies, but it would be nice if there were some discussion of them in terms of practical uses for this approach. More specifically germane to the results from the study:

1. Did naltrexone therapy have any effect on lidocaine analgesia alone?
2. Referring to Fig. 3A and the discussion in the text, was the naltrexone administered in the same dose, by the same route and at the same time as was indicated in Fig. 5? This should be stated. More importantly, the authors state that the synergistic analgesic effect of lidocaine/morphine was significantly blocked by naltrexone, but they don't say how much; the % of animals examined, analgesic after naltrexone should be stated.
3. In fig 5, Rincomb curves are included in both graphs and appear to be the same. At some point in this paper the authors should show the effects of naltrexone alone in this paradigm. One would be to exclude lidocaine from one of these graphs and insert the naltrexone curve instead.
4. On page 13 the authors state, "The activity of exogenous and endogenous opioids at the activity of opioid systems beyond two receptors." Are they referring to opioid systems with peripheral and central administration. Additionally, while Leu-enkephalin does affect action through mu or opioid receptors, the results from these studies don't really address this point. The fact that a single dose of naltrexone totally blocked Rincomb analgesia suggests that Leu-enkephalin was acting solely through a receptors, since its antagonism is less sensitive to naltrexone. In the absence of more selective μ antagonist antibodies (e.g. β-FNA), the authors should avoid making such blanket statements.

Please attach

Journal of Pharmacology and Experimental Therapeutics

Reviewer #2
Associate Editor: Dr. Rocha

MS #: JPET/2006/009875

MS Title: Analgesic synergy between topical lidocaine and topical opioids

Authors: Kosharskyy, Cherkasov and Pasternak

This article is an extension of recently-completed studies performed by the authors examining the analgesic response following topical administration of opioids in connection with other pharmacological agents. In this study, the authors perform straightforward and quite unambiguous demonstrations that topical lidocaine and topical opioids each produce analgesic responses alone, and display quite marked synergy following combined administration. It is very surprising given the advanced state of the field of analgesia that such studies have never been performed previously, but the authors convincingly demonstrate this important property of both drug classes. There are a number of comments and items that should be addressed.

1. p. 2, line 8: "...lidocaine with a low dose of an opioid..."
2. p. 6: Topical administration. The authors should provide a rationale/rationale as to why a DMSO solution was used.
3. p. 6, results and figure 1a: Indicate what dose of lidocaine was used in this particular initial experiment.
4. p. 9, lines 1 and 2: What dose and route of naloxone was used to reverse the analgesic effects of lidocaine and morphine?
5. p. 11, line 3: delete "as".
6. p. 11, line 10: "of Active opioids..."
7. p. 11, 1st sentence of third paragraph: The sentence does not make sense as presently constructed.
8. p. 12, line 11: "resulted from its receptor activity..."
9. p. 12, line 14: "...study, it will be of interest..."
10. The paper is devoid of any statistical data; it is up to the Editor if such additional data are necessary.

Minor errors:

1. Page 5, line 3 should read "housed" instead of "housing."
2. Page 12, line 22 – remove the word "of."
3. Page 13, line 14 – add the word "be" : "it will be substituted..."
4. Fig. 5A legend, line 4 should read "application was found to the cellular assay."
5. Fig. 6A, Y-axis label misspelled.
6. Fixed ratio listed in Fig. 6 legend do not jive with fixed ratios given in Table 1 - which is correct? Also, Fig. 6A legend should be in reference to bifunctional ratio, not heterocyclic.

148-23

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Analgesic Synergy between Topical Lidocaine and Topical Opioids[1]

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ABSTRACT

Topical drugs avoid many of the problematic side effects of systemic agents. Immersion of the tail of a mouse into a solution of dimethyl sulfoxide (DMSO)-containing morphine produces a dose-dependent, naloxone-sensitive, analgesia (ED_{50} 6.1 mM; CL 4.3, 8.4) limited to the portion of the tail exposed to the drug. DMSO alone in this paradigm had no analgesic activity. Like morphine, the opioids levorphanol (ED_{50} 5.0 mM; CL 3.8, 7.8) and buprenorphine (ED_{50} 1.1 mM; CL 0.7, 1.5) were effective topical analgesics. Lidocaine also was active in the tail-flick assay (ED_{50} 2.5 mM; CL 2.0, 3.4), with a potency greater than morphine. As expected, the free base of lidocaine was more potent than its salt. Combinations of a low dose of lidocaine with a low dose of an opioid yielded significantly greater than additive effects for all opioids tested. Isobolographic analysis confirmed the presence of synergy between lidocaine and morphine, levorphanol and buprenorphine. These studies demonstrate a potent interaction peripherally between opioids and a local anesthetic and offer potential advantages in the clinical management of pain.

Topical treatments offer many advantages over systemic drugs. By limiting the exposure of a drug to the periphery, central side effects can be markedly reduced. For opioids, this might decrease limiting side effects, such as sedation, respiratory depression, and nausea. Further limiting the drug to the actual site of action has even more advantages, by avoiding peripherally mediated side effects, such as constipation. In earlier studies, we demonstrated the activity of topical morphine in the radiant heat tail-flick assay after immersion in a dimethyl sulfoxide (DMSO) solution (Kolesnikov and Pasternak, 1999a). The analgesic actions seen with topical morphine were limited to the region of the tail exposed to the drug and were not seen in more proximal areas not exposed to the drug. DMSO alone was inactive in this paradigm. Other opioid ligands acting through kappa and delta receptors have activity peripherally in the radiant heat tail-flick assay as well (Kolesnikov et al., 1996a; Kolesnikov and Pasternak, 1999b). Thus, topical opioids might be useful in pain control.

Synergy is important in opioid action. First described between supraspinal and spinal sites (Yeung and Rudy, 1980), it has also been described between brainstem nuclei (Rossi et al., 1993) and between peripheral and central sites (Kolesnikov et al., 1996b). Synergy has been observed between opioids of different classes (Horan et al., 1992; Adams et al., 1993; Rossi et al., 1994; He and Lee, 1998).

Opioid actions also can be modulated by nonopioid classes of drugs. For example, opioid tolerance can be prevented or reversed by *N*-methyl-D-aspartate (NMDA) antagonists (Trujillo and Akil, 1991; Ben-Eliyahu et al., 1992; Tiseo and Inturrisi, 1993; Elliott et al., 1994) and nitric oxide synthase inhibitors (Kolesnikov et al., 1993, 1995). Unfortunately, NMDA antagonists have proven difficult to use systemically due to their profound psychomimetic and dysphoric actions. These problems might be avoided by a topical approach. We were able to demonstrate in our topical paradigm that the combination of an NMDA antagonist with an opioid blocked tolerance to the opioid (Kolesnikov and Pasternak, 1999a,c). This activity of NMDA antagonists topically presumably would avoid the limiting side effects that preclude their use systemically.

Lidocaine, a local anesthetic, is active topically, by blocking sodium channels, a mechanism distinct from the opioids (Woolsey and Funck-Brentano, 1988). Clinical studies have shown advantages to the combination of intrathecal lidocaine and opioids (Atanassoff et al., 1997; Saito et al., 1998a,b), leading us to question whether similar advantages might be seen topically. We therefore have examined the activity of topical lidocaine in the tail-flick assay alone and in combination with a number of opioids.

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ABBREVIATIONS: DMSO, dimethyl sulfoxide; NMDA, *N*-methyl-D-aspartate; CL, confidence limits.

Materials and Methods

Male CrlCD-1(ICR)BR mice (25–30 g; Charles River Breeding Laboratory, Bloomington, MA) were maintained on a 12-h light/dark cycle with food and water available ad libitum. Mice were housed in groups of five until testing. Opioids were generously provided by the Research Technology Branch of the National Institute on Drug Abuse (Rockville, MD). Lidocaine base was purchased from Sigma Chemical Co. (St. Louis, MO). Lidocaine base was used in all experiments unless indicated otherwise.

Topical Administration. Drugs were administered topically and analgesia assessed as previously described (Kolesnikov and Pasternak, 1998a). In this procedure, the distal portion of the tail (2–3 cm) is immersed in a DMSO solution containing the indicated drugs for the stated time, typically 2 min (Kolesnikov and Pasternak, 1999a). Prior studies have documented that DMSO alone has no effect when tested in this manner in the radiant heat tail-flick assay (Kolesnikov and Pasternak, 1999a). Furthermore, DMSO provides an effective way of exhibiting a wide range of drugs and facilitating their transport through the skin. The onset of analgesia is rapid, with peak effects seen immediately after the removal of the tail from the treatment solution. Therefore, we tested animals immediately after termination of topical administration.

Radiant Heat Tail-Flick Test. Testing was performed on the position of the tail immersed in the treatment solution, because the analgesic actions of agents administered in this manner are restricted to the exposed portions of the tail; proximal regions are not affected (Kolesnikov and Pasternak, 1998a). Antinociception, or analgesia, was defined quantitatively as a tail-flick latency for an individual animal that was twice its baseline latency or greater. Baseline latencies typically ranged from 2.5 to 3.0 s, with a maximum cutoff latency of 10 s to minimize tissue damage in analgesia-naive animals. Group comparisons were performed with the Fisher's exact test. ED_{50} values were determined with the Hilo program (Tallarida et al., 1978; Ussing and Jahnsen, 1981), as previously reported (Kolesnikov et al., 1998a).

Drug Interactions. Isobolographic analysis was used to determine drug interactions (Tallarida et al., 1997). ED_{40} values were determined for each agent alone. They were then tested together at various doses at a constant ratio based on their respective ED_{40} values. In the figures, all points represent ED_{40} values. Values on the axes represent the ED_{40} values for the indicated drug alone, and the line connecting them corresponds to simple additive interactions. Points lying below the line of additivity indicate synergism. Significance was assessed by the lack of overlap of the confidence limits of the combination value with the confidence limits of the line of additivity.

Results

Topical Lidocaine and Morphine Interactions. First, we assessed the activity of topical lidocaine using the same administration paradigm previously shown active for opioids and NMDA antagonists (Kolesnikov and Pasternak, 1999a). Earlier studies emphasized the importance of exposure time in the activity of morphine. Similarly, the analgesic response to lidocaine was dependent on the exposure time (Fig. 1A). The response from a constant concentration of lidocaine increased from 20% at 30 s to 70% at 2 min. Time action-curves revealed a maximal response immediately after removal of the tail from the solution, with a gradual decrease to baseline levels within 20 min (Fig. 1B). This response was slightly shorter in duration than a morphine dose giving the same maximal response. A lower lidocaine dose gave both a decreased maximal response and a shorter duration of action.

Both the free base and salt of lidocaine were examined

Fig. 1. Time dependence of topical lidocaine analgesia. A, groups of mice (n ≥ 10) were exposed to a fixed concentration of topical lidocaine (4.3 mM) for 30 s, 1 min, and 2 min and then were tested in the tail-flick assay immediately after termination of drug exposure. B, groups of mice (n ≥ 10) were treated with lidocaine (4 or 2.15 mM) or morphine (25 mM) for 2 min and then tested in tail-flick assay at the indicated time over 30 min.

(Fig. 2). Both were active, but the salt was less effective and plateaued at a 50% to 60% response. As expected, the free base form of lidocaine was more active, achieving a 75% response. However, it displayed a biphasic dose-response curve, with increases in concentration beyond 20 mM reversing a progressive lowering of analgesic activity. Morphine also was active, as previously reported (Kolesnikov and Pasternak, 1999a), with a potency intermediate between the two

Fig. 2. Effects of topical lidocaine and morphine. Groups of mice (n ≥ 10) were exposed to the indicated concentration of the free base of lidocaine, lidocaine HCl, or morphine for 2 min and tested immediately afterward.

TABLE 1

Analgesic potency of lidocaine and opioids alone and in combination

ED_{50} values were determined from dose-response curves and presented with 95% confidence limits. For lidocaine, the ED_{50} value was determined only from the initial portion of the curve. Combinations were also examined using increasing doses of a fixed ratio of the indicated drugs. ED_{50} values were determined and presented with the 95% confidence limits. The relative potency of the various drugs in combination were compared with the same drug alone as a ratio. The fixed ratios were as follows: lidocaine/morphine, 0.5; lidocaine/buprenorphine, 2.4; lidocaine/levorphanol, 0.6.

Treatment	Lidocaine		Opioid	
	ED_{50} Value	Ratio	ED_{50} Value	Ratio
	mM		mM	
Lidocaine alone	2.5 (2.0, 3.4)			
Morphine alone			6.1 (4.3, 8.4)	
Buprenorphine alone			1.1 (0.7, 2.5)	
Levorphanol alone			5.0 (3.8, 7.8)	
Lidocaine/morphine	0.55 (0.6, 1.1)	2.9	1.7 (1.2, 2.9)	3.6
Lidocaine/levorphanol	0.47 (0.3, 0.8)	5.3	0.94 (0.6, 1.6)	5.3
Lidocaine/buprenorphine	0.44 (0.3, 0.8)	5.7	0.18 (0.12, 0.246)	6.1

forms of lidocaine (Table 1). The antagonist naloxone given alone was without effect.

Initially we assessed potential interactions between lidocaine and morphine using a fixed, low dose of each (Fig. 3A). Alone, lidocaine and morphine produced peak responses of only 30%. Together, their peak response was 80%, far greater than anticipated from simple additive interactions ($P < .004$). Comparing the areas under the curve gave even more dramatic differences. As anticipated, naloxone (1 mg/kg; s.c.), given 20 min before agonist treatment, completely reversed the effects of the combination (data not shown).

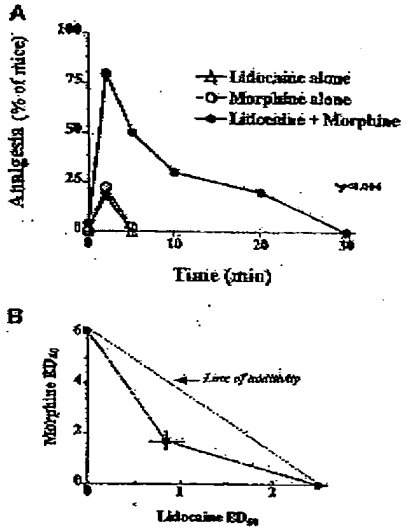

Fig. 3. Typical lidocaine and morphine interactions. A, groups of mice received either topical morphine (1.5 mM; $n = 10$) or lidocaine (0.9 mM; $n = 10$) alone or both together ($n = 20$). The combination was significantly ($P < .004$) more active at peak effect than the sum of two individual agents. B, using a fixed lidocaine/morphine ratio of 0.5, the ED_{50} value of combination was determined with the 95% confidence limits. The presence of the ED_{50} value below the line of additivity indicates the presence of synergy, confirmed by the lack of overlap between the 95% confidence limits for the drugs.

To further assess the possibility of synergy, we next employed isobolographic analysis (Tallarida et al., 1997). A dose-response curve was generated using increasing doses of a fixed ratio of lidocaine/morphine. The ED_{50} value fell well below the line of additivity, indicating synergism (Fig. 3B). The lack of overlap of the confidence limits of the combination value with those of the line of additivity confirmed its significance.

We also explored the relationship of lidocaine and morphine combinations by defining the ED_{50} values of each agent alone and in combination with a fixed dose of the other rather than a ratio (Table 2). Low doses of morphine with little activity alone markedly enhanced the potency of lidocaine. The effect seemed to plateau, with little advantage seen by increasing the morphine concentration from 3 to 4.5 mM. Similar results were seen with the morphine dose-response curves. Again, a low dose of lidocaine facilitated morphine analgesia, with little additional effect seen after doubling the lidocaine concentration from 0.6 to 1.8 mM. Thus, the enhanced activity of the combination of the drugs was most evident at low concentrations of each.

Topical Lidocaine and Other Opioids. We next explored whether the synergy seen with morphine/lidocaine combinations extended to other opioids with other receptor mechanisms of action, including levorphanol and buprenorphine. Typically, levorphanol and buprenorphine both yielded full analgesic responses, with ED_{50} values of 5.0 and 1.1 mM, respectively (Fig. 4; Table 1).

TABLE 2

Effects of fixed doses of morphine or lidocaine on the other's analgesic potency

ED_{50} values with 95% confidence limits were determined from at least three doses of topical lidocaine alone or with the indicated morphine concentration, or with topical morphine alone or with the indicated concentration of lidocaine.

	ED_{50}	95% Confidence Limits	Shift
	mM		
Lidocaine alone	3.5	(2.0, 3.4)	
+Morphine 1.5 mM	1.0	(0.4, 1.8)	2.5
+Morphine 3.0 mM	0.8	(0.6, 1.1)	3.1
+Morphine 4.5 mM	0.7	(0.5, 0.9)	3.6
Morphine alone	6.1	(4.3, 8.4)	
+Lidocaine 0.45 mM	3.6	(2.6, 4.5)	1.7
+Lidocaine 0.9 mM	1.5	(0.9, 2.6)	4.1
+Lidocaine 1.8 mM	1.3	(0.6, 1.9)	4.7

Fig. 4. Effects of topical buprenorphine and levorphanol. Groups of mice ($n \geq 10$) were exposed to the indicated concentration of the drug for 2 min and were tested immediately afterward.

Combinations of low doses of lidocaine and these opioids gave greater than additive analgesic actions (Fig. 5). The results with levorphanol closely resembled those of morphine, with the combination of low lidocaine and levorphanol doses giving a maximal response far beyond that expected by simple additive interactions ($P < .03$) as well as a prolonged duration far exceeding that of either alone (Fig. 5A). Although each drug alone had no effect beyond 5 min, together their response lasted for greater than 20 min. The effects of the combination of doses were readily antagonized by naloxone. The response to lidocaine alone (2.5 mM) was insensitive to naloxone (1 mg/kg, s.c.). (Data not shown.)

Buprenorphine and lidocaine gave similar results. The maximal responses of the two together were far beyond those anticipated by simple additive interactions (Fig. 5B). The duration of the responses of the combination also markedly differed from that of either agent alone. Alone, each drug lasted less than 10 min. In contrast, the duration of the response of the combination was prolonged. The peak effect of the combination was 80% and persisted for 10 min. Analgesia could still be demonstrated after 45 min. Indeed, the duration of this response from the lidocaine/buprenorphine combination exceeded that seen with any of the other opioids tested. Naloxone significantly lowered the response of the combination.

Fig. 5. Effects of combinations of low doses of opioids with lidocaine. A, groups of mice ($n = 20$) received intrathecal lidocaine (0.6 mM) or levorphanol (1.8 mM) or the combination of the two for 2 min and were tested in the tail-flick assay over 90 min. Another group of mice ($n = 10$) received naloxone (1 mg/kg, s.c.) 20 min before the topical drug application and was tested in tail-flick assay. Naloxone significantly reduced the response. B, groups of mice ($n = 20$) received either topical lidocaine (0.6 mM) or buprenorphine (0.5 mM) or the combination of the two for 2 min and then were tested in the tail-flick assay over 60 min. Another group of animals received naloxone (1 mg/kg, s.c.) 20 min before the topical drug application. Naloxone significantly reduced the response.

Isobolographic Analysis of Lidocaine/Opioid Interactions. We next examined the combinations of the additional opioids isobolographically using dose-response curves with fixed ratios of the two drugs in combination (Fig. 6; Table 1). Combining levorphanol with lidocaine enhanced their relative potencies over 5-fold, which was more than the enhancement of morphine by lidocaine. Isobolographic analysis was consistent with synergy (Fig. 6A). Buprenorphine and lidocaine together shifted their individual ED_{50} values approximately 6-fold. Again, isobolographic analysis indicated synergy (Fig. 6B).

Discussion

Lidocaine is a widely used local anesthetic (Woolsey and Foreck-Brenemo, 1998). It acts through the blockade of sodium channels, a mechanism distinct from the opioids. In the current study, lidocaine was effective topically in the rodent heat tail-flick assay, working only on the portion of the tail exposed to the drug and with a potency greater than morphine. As anticipated, the free base was more effective than the salt, presumably due to its greater lipophilicity. However, its dose-response curve was biphasic, with concentrations greater than 20 mM giving a progressive decrease in response. The reasons for this are not clear, but it is interesting that lidocaine concentrations above 15 mM can be toxic to neurons in primary culture (Gold et al., 1998).

All of the opioids tested were effective topical analgesics. The activity of levorphanol and buprenorphine extends the activity to drugs working on opioid systems other than simply mu receptors. Levorphanol clinically analgesia through both mu and kappa$_2$ receptors (Mimbin et al., 1988; Tive et al., 1992). Buprenorphine has a complex mechanism of action that is not entirely clear (Leander, 1987; Kamai et al.,

Fig. 6. Isobolographic analysis of lidocaine interactions with levorphanol and buprenorphine. A, using a fixed lidocaine/levorphanol ratio of 15:5, the ED$_{50}$ values of the combination with the 95% confidence limits were determined from the dose-response curve. The point falls below the theoretical line of additivity between the ED$_{50}$ values for each drug alone, indicating synergy. The lack of overlap between the 95% confidence limits for the drugs alone and the combination implies the synergy is significant. B, using a fixed lidocaine/buprenorphine ratio of 2:4, the ED$_{50}$ values of the combination with the 95% confidence limits was determined from the dose-response curve. The point falls below the theoretical line of additivity between the ED$_{50}$ values for each drug alone, indicating synergy. The lack of overlap between the 95% confidence limits for the drugs alone and the combination implies the synergy is significant.

1996a,b, 1997; Wahler et al., 1996). Although it has high affinity for virtually all classes of opioid receptors in binding studies, it also has widely varying efficacies for the various classes of receptors. Topically, buprenorphine was particularly effective, with a potency 8-fold greater than that of morphine. The limited ability of naloxone to reverse the combinations of buprenorphine and lidocaine implies that at least a portion of the response from buprenorphine was invoked from non-mu-opioid receptors.

Opioid analgesic synergy has been well established. Initially, it was observed among regimens simultaneously exposed to opioid (Tsang and Rady, 1980; Rossi et al., 1993, 1994; Kolesnikov et al., 1996b), followed by the demonstration of synergy between different classes of opioids (Adams et al., 1998). Morphine also has been reported to demonstrate synergy with lidocaine centrally (Saito et al., 1998a,b). We now find synergy peripherally between topical opioids and a local anesthetic.

The combination of a low dose of morphine and lidocaine clearly revealed activity far beyond simple additive interactions, as did similar studies with the other opioids. These strongly suggested synergy among the opioids with lidocaine. This was not unexpected. Synergistic interactions might be more likely when drugs act on different mechanisms, as shown here with the opioids and lidocaine. Isobolographic analysis confirmed synergy between lidocaine and the opioids. The most impressive interaction was between buprenorphine and lidocaine, which had the greatest potentiation and the longest duration of action. However, it is not clear whether this resulted from its receptor selectivity or other factors such as its greater lipophilicity, which would enhance its ability to become diffused through the skin.

The demonstration of synergy between lidocaine and more than one opioid receptor ligand deserves more study. It will be of interest to define the opioid receptor mechanisms involved more clearly. However, even without a full understanding of how these agents interact, the demonstration of topical synergy between a local anesthetic and opioids opens many clinical possibilities in pain management.

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